

***Malamoeba indica* n.sp. from the Malpighian tubules of  
*Poecilocera picta***

C C NARASIMHAMURTI and S NAZEER AHAMED\*

Department of Zoology, Andhra University, Waltair 530 003, India

\* Present address : Govt. H. S. School, Pasighat, Arunachal Pradesh, India.

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**Abstract.** A new species of histozic amoeba, *Malamoeba indica* parasitic in the epithelial cells of the midgut and the lumen of the Malpighian tubules of *Poecilocera picta* is described. The trophozoites reach a maximum size of  $13.5 \times 7.0$  m and divide by binary fission after the endosome breaks down into chromatin masses. The cysts are ellipsoid with an outer thin refractile wall and measure  $14.5 \times 8.6$   $\mu$ m in the fresh condition and  $13.5 \times 7.5$   $\mu$ m in the fixed and stained condition.

**Keywords.** *Malamoeba indica*; *Poecilocera picta*; Malpighian tubules.

## 1. Introduction

While examining the sections of the midgut epithelium and the lumen of the Malpighian tubules of *Poecilocera picta* we came across a new rhizopod-parasite belonging to the genus *Malamoeba*. A perusal of the literature showed that so far only one species belonging to this genus has been reported from several species of grasshoppers from different parts of the world. The present form differs from it in several respects and hence is described as a new species in the present paper.

## 2. Material and methods

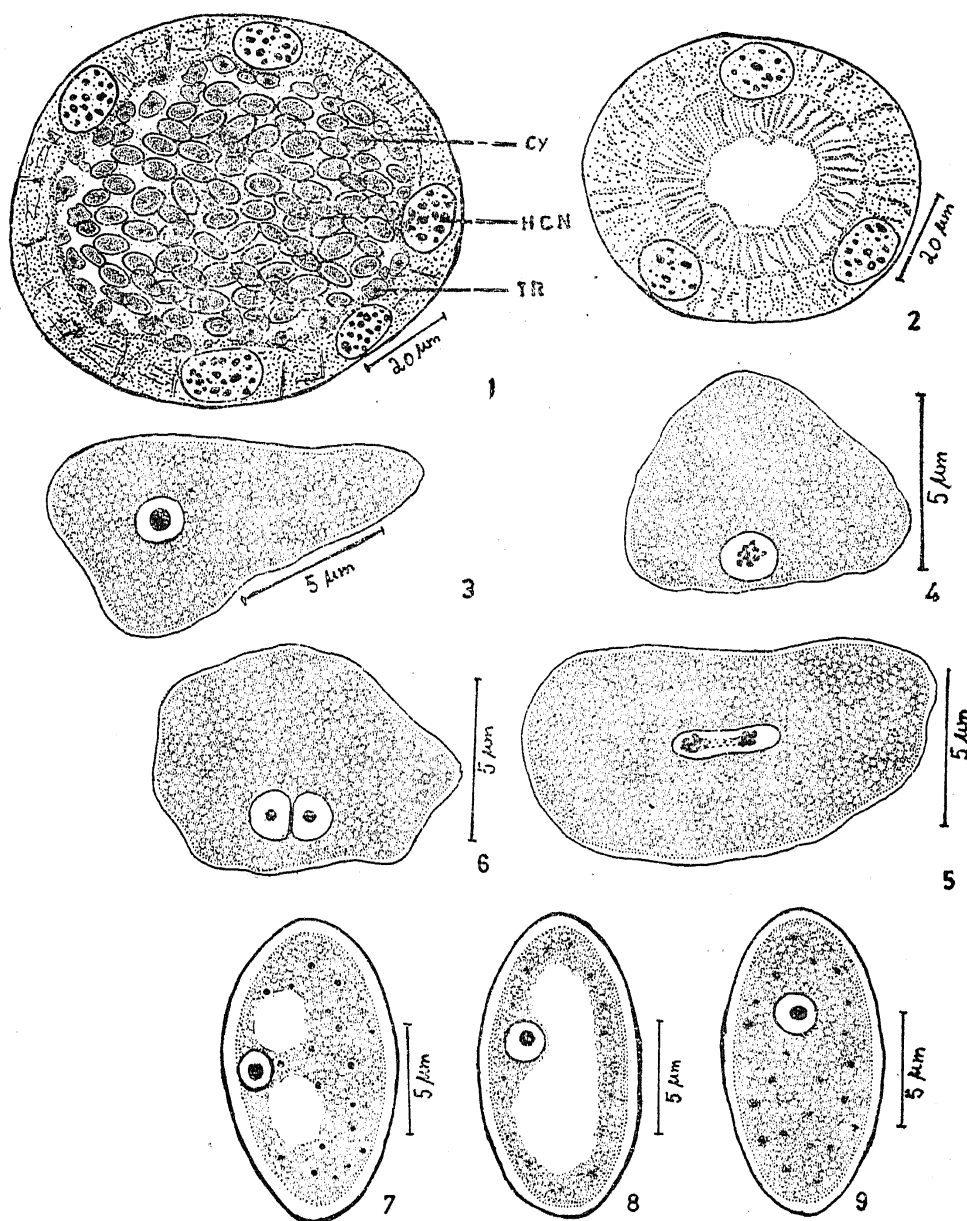
The painted grasshopper, *Poecilocera picta* occurs abundantly during April-December period feeding voraciously on the leaves of *Calotropes* and *Nerium*. There are no external indications of infection, but heavily infected forms were sluggish. Observations on fresh parasites were made by dissecting out the Malpighian tubules and examining them microscopically in Ringer's or normal saline under the pressure of a cover slip. Smears of the contents of the Malpighian tubules were either air-dried, fixed in methyl alcohol and stained in Giemsa or wet-fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin. Material for sectioning was fixed in alcoholic Bouin's fluid, sectioned at 8  $\mu$ m thickness and stained with iron haematoxylin.

### 3. Observations

About 5% of the grasshoppers examined were infected with amoeba. The trophozoites were found in the epithelial cells of the midgut and trophozoites and cysts were found in the lumen of the Malpighian tubules. Neither trophozoites nor cysts were found in the haemocoel. The trophozoites reach a maximum size of  $13.5 \times 7.0 \mu\text{m}$  (range:  $4.2\text{--}13.5 \times 4.0\text{--}8.0$ ; average  $9.27 \times 5.78$ ;  $n = 50$ ).

They lie in close contact with the inner wall of the Malpighian tubule or were inside the epithelial cells of the midgut. In heavy infections the tubules appeared swollen, the lumen being packed with the parasites (figure 1). The brush border was also destroyed (figures 1 and 2). In fresh condition the amoebae moved about by putting forth a single large pseudopodium. A few other smaller pseudopodia were also present but they apparently did not play any role in locomotion because the amoeba always moved in the direction of the larger pseudopodium. In stained preparations, the cytoplasm appeared finely alveolated and sometimes contained a few refringent granules scattered all over the cytoplasm. The nucleus which was rounded had a well-defined nuclear membrane and contained a single, large deeply-stained centrally-placed endosome. There was no extra-endosomic chromatin material. In the dividing stages the single endosome divided into 8–10 chromatin bits, which later arranged themselves in two groups of 4 or 5 each and moved in the opposite directions, sometimes connected by an achromatic filamentous structure (figures 4 and 5). The next stage observed was an amoeba with 2 nuclei which were placed in close apposition (figure 6). The entire process of nuclear division appeared to take place with the nuclear membrane intact. Amoebae showing more than 2 nuclei were never observed and hence it is presumed that only binary fission is present in the present form. Evans and Elias (1970) who studied the life-cycle of *Malamoeba locustae* after experimentally infecting healthy male *Locusta migratoria migratoides* distinguished two types of morphologically distinct types, the primary ones which divide by a process of binary fission and the secondary trophozoites which are a result of multiple fission.

The cysts were ellipsoid with an outer thick refractile wall and measured  $14.5 \times 8.6 \mu\text{m}$  in the fresh condition and  $13.5 \times 7.5 \mu\text{m}$  in material fixed in methanol and stained with Giemsa. They were found in the lumen of the Malpighian tubules and are passed out along with the faecal matter. The cytoplasm was hyaline and contained a few refringent granules. The nucleus was present in the centre towards a side when vacuoles were present and in the middle when the vacuoles were absent. It measured  $3.2 \mu\text{m}$  in diameter and contained a single deeply stained endosome (figures 7, 8 and 9). A small quantity of extra-endosomic chromatin material was present in the form of fine granules adherent to the inner wall of the nuclear membrane. In some of the cysts 2 vacuoles were present, one at either pole (figure 7) while in some others only one vacuole was present which appeared to be the result of confluence of the 2 vacuoles and occupied a large space in the centre. Some of the cysts did not have any vacuoles (figure 9). The vacuole was stained light brown with Lugol's iodine and perhaps is comparable to similar vacuoles seen in *Entamoeba* and are of the nature of glycogen. It is possible that cysts without vacuoles are mature forms.



Figures 1-9. 1. T.S. of infected malpighian tubule showing lumen filled with trophozoites and cysts. 2. T.S. of an uninfected malpighian tubule. 3. A trophozoite. 4. Trophozoite with the endosome broken down to 8-10 bits. 5. Chromatin bits separated into two groups of 4-5 and moving in opposite directions. 6. An amoeba with 2 nuclei lying side by side. 7. Cyst with 2 vacuoles. 8. Cyst with 1 large vacuole. 9. Cyst without a vacuole. (Abbreviations : Cy, Cysts; HCN, Host cell nucleus; TR, Trophozoites.)

#### 4. Discussion

Prell (1926) established the genus *Malpighamoeba* to include *M. mellifica* from the honey bee, *Apis mellifica*. Later King and Taylor (1936) described *M. locustae* from the Malpighian tubules of 3 species of grasshoppers belonging to the genus *Melanoplus*. Taylor and King (1937) carried out further studies on *M. locustae* King and Taylor, 1936 and compared with *M. mellifica* Prell, 1926 and found that

they did not belong to the same genus, because the endosome in *Malpighamoeba mellifica* divided without fragmenting forming polar caps as in the case of *Vahlkampfia* whereas in the case of *Malpighamoeba locustae* the endosome disintegrated and chromosomes and fibrils are formed as in *Hartmannella*. Hence they proposed erection of a new genus *Malamoeba* and designated *M. locustae* described by them earlier (King and Taylor 1936) as the type species. They further observed that these parasites not only occur in the lumen of the Malpighian tubules as described by them earlier but are also found in the epithelial cells lining the midgut and caeca. The same amoeba was subsequently reported from 3 species of *Melanoplus* by Steinhaus and Marsh (1962), from *Locustina pardalina* (Walker) by Prinsloo (1960) and Venter (1966) from *Locusta migratoria migratoides*, by Evans and Elias (1970) and from *Chortoicetes terminifera* by Davies (1973) both in natural populations and from laboratory cultures from different parts of the world. Harry and Finlayson (1976) who studied the life-cycle, ultrastructure and the mode of feeding of the locust amoeba, *Malpighamoeba locustae* showed that the primary trophozoites excyst in the crop and midgut and then penetrate the epithelial cells of the midgut and caeca where they multiply slowly and from the lumen of the gut make their way into the lumen of the Malpighian tubules where they feed on the brush border as extracellular parasites. During the first few days they undergo a rapid series of divisions which enable them to double their numbers every 24 hr or so. They did not find any primary or secondary trophozoites in the haemocoel of the host contrary to what was stated by Evans and Elias (1970) that the primary trophozoites migrate through the walls of the gut into the haemocoel and from there migrate through the tubule walls to reach the lumen. We have also not found any evidence of the presence of either the trophozoites or cysts in the haemocoel and their subsequent migration through the walls of the Malpighian tubules into the lumen of the tubule.

In the present form the division of the nucleus resembles that of *Malamoeba* and the only species described so far is *Malamoeba locustae* from different species of locusts. The trophozoites in the present form reach a maximum size of  $13.5 \times 7.0 \mu\text{m}$  while in *Malamoeba locustae* originally reported by King and Taylor (1936) and Taylor and King (1937) measured  $5-10 \mu\text{m}$  in diameter. Steinhaus and Marsh (1962) who reported the parasite from 3 species of *Melanoplus* observed that the trophozoites were generally spherical ranging from  $4-12 \mu\text{m}$  in diameter in fresh preparations. The cysts in the present form measured  $14.5 \times 8.6 \mu\text{m}$  in the fresh condition and  $13.5 \times 7.5 \mu\text{m}$  in the fixed and stained condition. King and Taylor (1936) reported that the cysts in *M. locustae* averaged  $9.6 \times 5.5 \mu\text{m}$ . Prinsloo (1960) reported an average of  $12 \times 8 \mu\text{m}$ . Henry (1968) in an effort to determine the reason for variation in size of cysts measured them from individual specimens of *Melanoplus vittatus*, *M. differentialis* and *M. sanguinipes* reared between  $28^{\circ}-35^{\circ}\text{C}$ . Fresh cysts suspended in distilled water showed that they measured  $12.58 \times 7.72$ ;  $12.25 \times 7.58$  and  $12.63 \times 7.42 \mu\text{m}$  respectively and when fixed in methanol and stained with Giemsa solution measured  $11.52 \times 6.65$ ;  $11.27 \times 7.22$  and  $10.62 \times 6.14 \mu\text{m}$  respectively. It is thus seen that both the cysts and trophozoites in the present form are larger than that of the only form described. There is also no evidence of occurrence of two morphologically different types of trophozoites in the present form as was observed in *Malamoeba locustae* by Evans and Elias (1970). Further the present form is the first report from the present

host and from the present geographical locality. For these reasons the present parasite is considered a new species and the name *Malamoeba indica* n.sp. is proposed for the same.

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